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# THE SALTON SEA: THE ACTION OF SALTON SEA WATER ON VEGETABLE TISSUES

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# THE ACTION OF SALTON SEA WATER ON VEGETABLE TISSUES.

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The recession of the waters of the Salton Sea began in February 1907, and as the level fell from 42 to 54 inches annually, each year laid bare wide strips of beach which had been occupied by a halophytic and xerophytic vegetation previous to the formation of the lake. It is probable that many representatives of the species listed by Mr. Parish in a separate section of this volume were covered by the water during the years 1904 to 1907.

But examination of the emersed zones uncovered by the recession of the lake failed to disclose the remains of any annuals or herbaceous plants, and their disappearance may be attributed to facts revealed by the investigations reported in this paper. Remains of the woody plants were fairly abundant, however, and in the autumn of 1911 Dr. D. T. MacDougal forwarded portions of stems and branches of *Prosopis glandulosa* and *Larrea tridentata* to the Botanical Laboratory of the University of Chicago, where a study of the changes which this material had undergone was made. The material included specimens which had been submerged in 1906 and had emerged in 1907, 1908, 1909, 1910, and 1911. It was all thoroughly desiccated by the time it came to hand. The specimens were very hard, except those which had been submerged but a year, and all were destitute of cortex. (12 s., 13, and 14.) Material which had been submerged for more than a year had undergone the full extent of the alterations described below.

Efforts were made to secure literature relating to the subject under investigation, but extensive inquiries in America and Europe were fruitless. While a very large number of examinations have been made of the action of saline and brackish water on living plants, reported in hundreds of papers, and while many tests of the preservative effects of various salts on different woods have been made, yet the action of saline waters or fresh waters on the tissues of plants killed by flooding and remaining immersed *in situ* for long intervals of time has been little investigated. This omission seems strange when we consider the intimate relations of the physical chemistry of such problems and the large economic questions involved in the disposal of sewage, decomposition of manure and humus, and the carbonization processes associated with the coal formations.

The appreciation of the problem reported in this paper will be increased by knowledge of the geography and topography of the Salton Sea region. Detailed reports respecting the formation of the Colorado district and the low-lying basin known as the Salton Sea may be had by consulting references (1)[1] and (2),[2] and the sections of this volume on the geography and surface geology of the Salton Sink and the Cahuilla Basin.

A consideration of the physical and biological factors operating in the Salton Sea waters during the years that these woods were immersed, are illuminating in connection with experiments which were carried forward in the Botanical Laboratories of the University of Chicago while endeavoring to discover the processes which caused the changes in the woody tissues.

The chemical analyses of the Salton Sea waters during the years 1907–11 are instructive. The reports set forth in table 24 were secured by the chemists, Dr. W. H. Ross and Prof. R. H. Forbes.

[1] D. T. MacDougal: The Desert Basins of the Colorado Delta, Bull. Am. Geog. Soc., Dec. 1907.
[2] Newell: Smithsonian Report, pp. 331–345, 1907.

TABLE 24.

| Total solids (dried at 110° C.) | June 3, 1907 | May 25, 1908 | June 8, 1909 | June 3, 1910 | June 3, 1911 |
|---|---|---|---|---|---|
| Plus $H_2O$ of occlusion and hydration | 364.80 | 437.20 | 519.40 | 603.80 | 718.0 |
| Sodium | 111.05 | 134.26 | 160.33 | 189.28 | 227.81 |
| Potassium | 2.29 | 2.78 | 3.24 | 3.53 | 3.81 |
| Calcium | 9.95 | 11.87 | 13.70 | 13.67 | 15.62 |
| Magnesium | 6.43 | 7.63 | 8.96 | 9.84 | 11.68 |
| Aluminium | 0.030 | 0.35 | 0.62 | 0.040 | 0.089 |
| Iron | 0.005 | 0.006 | 0.010 | .008 | 0.036 |
| Manganese, none any year | ..... | ..... | ..... | ..... | ..... |
| Zinc, none any year | ..... | ..... | ..... | ..... | ..... |
| Lead, none any year | ..... | ..... | ..... | ..... | ..... |
| Copper | ..... | ..... | trace | trace | trace |
| Lithium | trace | 0.013 | 0.017 | .021 | 0.025 |
| Chlorine (Cl) | 169.75 | 204.05 | 240.90 | 280.93 | 339.42 |
| Sulphuric acid ($SO_4$) | 47.60 | 56.74 | 65.87 | 76.36 | 91.67 |
| Carbonic acid ($CO_3$) | 6.58 | 7.66 | 7.34 | 6.38 | 5.78 |
| Silicic ($SiO_3$) | 1.41 | 1.43 | 1.59 | 1.55 | 1.83 |
| Phosphoric ($PO_4$) | 0.009 | 0.011 | 0.01 | .013 | trace |
| Nitric ($NO_3$) | 0.18 | 0.20 | none | none | none |
| Nitrous ($NO_2$) | none | trace | 0.0006 | none | none |
| Oxygen consumed | 0.093 | 0.059 | 0.068 | .045 | 0.063 |
| Boric acid | ..... | trace | ..... | trace | trace |

A critical examination of the substances present in the Salton Sea waters does not indicate that they are active in disintegrating the cell walls of woody tissues or necessarily cause even a breaking down of the tissues in herbaceous plants. However, the sulphates present in the water have a very definite relationship to certain bacteriological processes supposed to accomplish decomposition of cortical portions of woody stems.

No analyses were made with reference to the gaseous contents of the Salton Sea water, but it contains manifestly an abundance of oyxgen, inasmuch as large numbers of fish were present during the summer of 1912. The water has a specific gravity of 1.001. It is clear and quite free from sediment and may show an osmotic pressure of 5.5 atmospheres. The currents of the water are largely confined to those produced by the wind, since there is no outlet to the basin. A review of the physical facts indicates that the decomposition of vegetable tissue immersed in the water, even though some of the woods were covered for five years, could not be attributed to the action of non-biological agents. This conclusion was supported by the laboratory tests conducted with fresh woody tissues of *Prosopis glandulosa*, *Prosopis pubescens*, and *Larrea tridentata*, which remained absolutely intact during the eight months of submergence in sterilized Salton Sea water.

Biologically considered, the submerged condition of all of the herbaceous and woody plants would be, of course, fatal to those organisms within a short time and would remove them, therefore, entirely from the question of resistance to the physico-chemical factors of the Salton Sea. On the other hand, the temperature, the light, the gas-content, and the food supply were extremely favorable for the development of a rich bacterial flora. This was demonstrated by the fact that there were several thousand bacteria per cubic centimeter of water when first collected from the Salton Sea. These organisms were represented by several species, some of which were found to have a definite relation to the change in the chemical composition of the water and to the decomposing processes undergone by dead, submerged organisms. Inasmuch as the plankton did not seem to enter into this study all reference to that biological phase of the Salton Sea was omitted.

The specific investigations of this problem were directed (1) to an anatomical study of the species submerged from one to five years, and (2) to a bacteriological study of considerable quantities of the water itself and of processes undergone by fresh woods submerged in containers filled with Salton Sea water and maintained at room temperature in the Botanical Laboratory. The investigations concluded with an anatomical investigation of the fresh woods which had been kept under control in the laboratory during the months that the bacteriological experiments were carried forward.

A

B

C

A. Prosopis glandulosa, the base of which was submerged in 1906 and laid bare late in 1907. Photographed late in 1908 by D. T. MacDougal.

B. Segment of Crescentic Dune surrounded by Rising Waters of Lake in vicinity of Carrizo Creek, February 1907.

C. Tongues of Water from the Rising Sea in the Channels of Desert Washes, Imperial Junction Beach, February 1907.

## ANATOMICAL STUDIES OF THE SPECIMENS SUBMERGED ONE TO FIVE YEARS.

Obviously it was important to learn definitely what changes, if any, had taken place in the tissues of these woody plants during their term of submergence, as indicated in Plates 13 and 14L. It was found that almost the entire cortex had been lost from all specimens except those which emerged in 1907, after a single year's immersion. A detailed study of the cell walls was necessary in order to answer a question which had arisen relative to the possible procedure of petrifaction. In addition, to give opportunity for study of anatomical structures, a series of sections were made in the transverse, tangential, and radial planes. These were placed in various liquids in order to soften them for sectioning on a special type of swinging microtome. Sterile water, unsterile water, and concentrated hydrofluoric acid were used. It was impossible to secure sections thinner than 25 microns in any of the specimens not freed from mineral contents by hydrofluoric acid. In the specimens that were treated from one to three weeks in concentrated hydrofluoric acid and thoroughly washed it was possible to section 5 to 10 microns. In all cases the sections prepared from the water or hydrofluoric acid softened specimens were stained with a water solution of safranin for 24 to 36 hours and counterstained with aniline blue for a few seconds. These sections showed entire absence of epidermis, cortex, and phloem. The woody cylinder was unchanged in the xylem, ray, and pith regions. These conditions were the same in both *Prosopis* and *Larrea.*

Microscopical measurements of the walls in the cells of the ray regions and pith and the xylem were made in order to determine whether there were variations in the tissues of the woods that had been submerged one, two, three, four, and five years. In no case was there any difference in the same region of the same plant. The walls of the ray cells were 1.9 to 2.5 microns thick; those of the wood fiber cells were 5.7 to 6.6 microns, and those of the tracheæ were 5.5 to 9.5 microns for *Prosopis glandulosa;* for the same regions of *Larrea* they were 1.4 to 2.3 microns, 0.9 to 4.8 microns, and 5.5 to 6.5 microns thick, respectively.

Sections of the woods softened in water preparatory to cutting were carefully examined for evidence of mineral deposition. In no case could crystals other than calcium oxalate be found. These crystals were present in the same relative places in the sections of the fresh woods and in similar quantities as they were in the dried woods that had been submerged. In view of this situation, one was forced to conclude that petrifaction had not been initiated in any of the woods submitted for study.

Since the samples of wood that were sent from the Salton Sea represented the final stages of the decomposition processes which had decorticated them it was thought best to secure water from the Salton Sea and reproduce, so far as possible, what had taken place in nature when the samples forwarded underwent decortication. Eight 5-gallon carboys of Salton Sea water were sent to Chicago, four of which were labeled (A), (B), (C), and (D). Pieces of fresh *Prosopis glandulosa* were placed in (A), of fresh *Prosopis pubescens* in (B), and of fresh *Larrea tridentata* in (C); and fresh specimens of all three woods were placed in (D). These cultures were started on December 9, 1911. The tightly stoppered carboys were maintained at a temperature of 22° C. After five days, there was a pronounced odor of hydrogen sulphide when the stoppers were removed from the carboys. A milky condition developed in all of the containers supplied with fresh woods. Carboy (C), containing *Larrea tridentata,* soon showed a remarkable growth of white threads festooned just below the neck of the carboy. These proved to be growths of *Beggiatoa,* which is semi-anaërobic. The threads were neither on the surface nor at the bottom of the carboy, but in an intermediate position with reference to the oxygen supply (Plate 14M). Later, white films formed on the surface of carboys (A), (B), and (D). A careful examination of these showed that they were composed of filaments of *Beggiatoa* and free sulphur liberated by the *Beggiatoa* when they used the hydrogen sulphide for energy releasal in

place of free oxygen. According to Omelianski,[1] *Beggiatoa* is dependent upon the action of *Spirillum desulphuricans* (which reduces the sulphates of mineral waters and liberates the hydrogen sulphide that serves the *Beggiatoa* for food supply), and the hydrogen sulphide is acted upon by an organism that oxidizes it and forms sulphuric acid. It was found that this acid acted upon iron and formed ferrous sulphide which dropped to the bottom of all of the carboys in considerable quantities as the foregoing processes proceeded in the carboys of Salton Sea water. Chemical analyses of water from the various carboys were secured through the kindness of Dr. Julius Stieglitz, of the Department of Chemistry of the University of Chicago. (Table 25.) Carboys (A) and (B) contained Salton Sea water, while carboys (C), (D), and (E) contained Salton Sea water into which specimens of *Prosopis glandulosa*, *Prosopis pubescens*, and *Larrea tridentata* respectively had been placed six months previously.

TABLE 25.—*Analyses of water of Salton Sea, in grams per 100,000 c.c.*

| | (A) | (B) | (C) Prosopis glandulosa. | | (D) Prosopis pubescens. | (E) Larrea tridentata. |
|---|---|---|---|---|---|---|
| | | | No. 1 | No. 2 | | |
| Chlorine (Cl)..... | 394.735 | 397.512 | 400.146 | 401.100 | 397.040 | 393.760 |
| Sulphuric ($SO_4$)..... | 105.233 | 107.650 | 75.96 | 75.39 | 101.056 | 105.800 |
| Hydrogen sulphide ($H_2S$).. | ..... | ..... | 1.9979 | 1.0718 | .6088 | .08616 |

In the waters which contained an appreciable quantity of hydrogen sulphide there was a decided decrease in the amount of sulphate ion, showing that the $SO_4$ had been reduced. From the chemical analyses it is shown that every 100,000 of the parts of the Salton Sea water contain from 105.233 to 107.650 parts of sulphate ion before receiving the different woods. After retaining the wood specimens for six months the amount of sulphuric ($SO_4$) varied from 105.800 in *Larrea tridentata* to 75.39 parts in the culture of *Prosopis glandulosa*. It was necessary to determine whether or not these changes might have resulted from the direct action of substances in the water.

According to the work of Beijerinck[2] this reduction of sulphates may have been accomplished by micro-organisms. One of the prominent anaërobic forms is *Spirillum desulphuricans*. He later[3] discovered forms that were less definitely anaërobic, which were able to aid in sulphate reduction. The final products of this bacterial action include sulphureted hydrogen as one of the main constituents. Thus we have an explanation of the large quantities of hydrogen sulphide that appeared in the cultures of *Prosopis* and *Larrea* in Salton Sea water. There were similar but less pronounced results in the cultures of these same woods in Lake Michigan water, and similarly the same when *Robinia pseudacacia* was placed in Salton Sea water and Lake Michigan water cultures.

The next modification in the chemical change of the output of these first bacterial forms is caused by *Beggiatoa*, according to Winogradsky.[4] This organism uses the hydrogen sulphide as a source of energy releasal. The hydrogen sulphide is oxidized to sulphuric acid, and free sulphur is first stored in the cell and then liberated in a free condition. The sulphuric acid acts upon the carbonates or some of the bases, as iron, and forms sulphates and ferrous sulphide. This accounts for the large amounts of free sulphur which collected in the bottom of all of the culture carboys of Salton Sea water. It also explains why considerable ferrous sulphide collected in the bottom of the vessel in which the greatest amount of sulphuric was decomposed, *i.e.*, *Prosopis glandulosa*, where the 105.233 parts per 100,000 of Salton Sea water were reduced to 75.39 parts after the $H_2S$ was largely oxidized.

[1] Omelianski, W., (1) Centralbl. f. Bakt. 2 Abt., vol. VIII, p. 193, 1902; (2) vol. XI, p. 369, 1904; (3) vol. XII p. 33, 1904.
[2] Beijerinck: Centrbl. Bakt., vol. II, 1, p. 1, 1895. [3] Beijerinck: Centrbl. Bakt., vol. II, p. 193, 1895.
[4] Winogradsky: Ueber Schwefelbacterien, Bot. Ztg., vol. LIV, p. 493, 1887.

## Details of Plate 13.

Figs.

A. *Prosopis glandulosa* submerged in the Salton Sea 1906 to 1908. Only the wood cylinder was left in this specimen, the cortex and phloem having been entirely decorticated.

B. *Prosopis glandulosa* after continuous submergence for two months in Lake Michigan water. Very little decortication had taken place at the completion of this period.

C. Specimens shown in B after two months more of submergence in Lake Michigan water. Slight decortication is shown in the two middle transverse sections.

D. Sections of *Prosopis glandulosa* after two months of submergence in Salton Sea water. The longitudinal sections show far more decortication than was indicated in B, the specimens that had been submerged an equal time in Lake Michigan water.

E. Four of the specimens shown in D have now advanced greatly in decortication during four months more of submergence in Salton Sea water. All of the phloem and cortex is raised from the ends of the two long sections and entire decortication has occurred in the two small transverse sections. Hydrolysis has advanced far in the cambial regions of the sections which have been submerged constantly for six months in Salton Sea water.

F. *Prosopis glandulosa* in transverse section. This shows the cortex (*c*), phloem (*p*), and xylem (*x*). In the phloem (*p*) the zones of alternating lignified (*lig.*) and unlignified (*unlig.*) tissue are shown. Hydrolysis of the unlignified (*unlig.*) tissue here coincides with the broken-down layers in D and E where the lignified (*lig.*) tissue is lifted in well-defined layers, after the unlignified (*unlig.*) tissue was hydrolyzed.

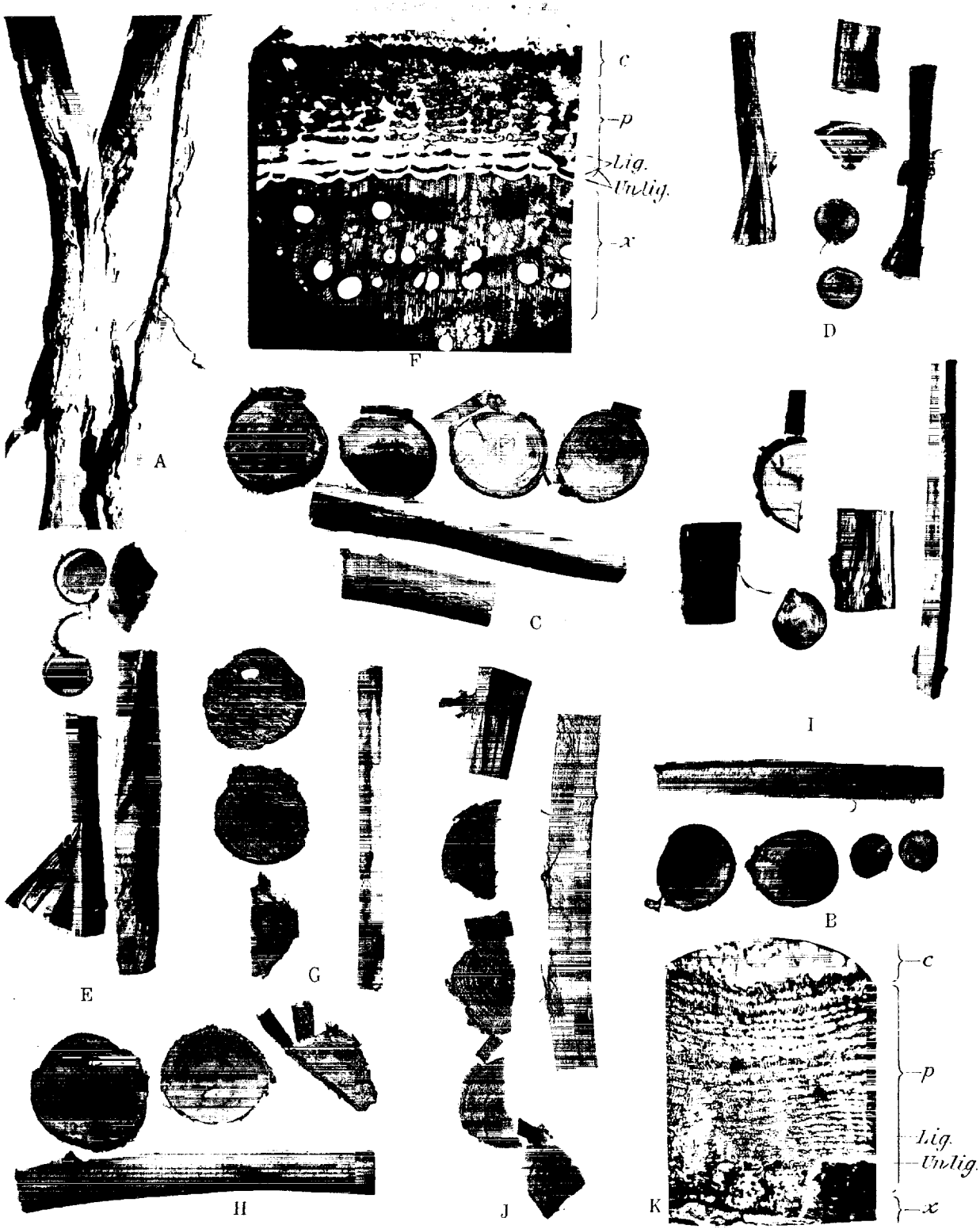
G. *Prosopis pubescens* photographed after two months of submergence in Lake Michigan water. Decortication is slight as in B.

H. Same culture as G after two months of further submergence in Lake Michigan water. The upper transverse section shows considerable decortication after four months of submergence in the Lake Michigan culture.

I. *Prosopis pubescens* after two months of submergence in Salton Sea water. Some decortication is shown in one transverse section.

J. Same culture as shown in I after two months more of submergence in Salton Sea water. Marked decortication is indicated by the four transverse sections.

K. *Prosopis pubescens* showing the cortex (*c*), phloem (*p*), and xylem (*x*) in an interesting relation when compared with F. Here closely associated lignified (*lig.*) zones are separated by very thin unlignified (*unlig.*) regions, consequently *Prosopis pubescens* does not decorticate after the manner of *Prosopis glandulosa*. A comparison of K and F, and comparisons of B and C with G and H, also a comparison of D and E with I and J are instructive.




Prosopis glandulosa and Prosopis pubescens in various Stages of Decortication. The Water Cultures in this work were maintained at Room Temperature of 22° C.

It is not understood that these analyses represent the chemical conditions of cultures, in which the various woods were placed, during the entire time of the investigation, inasmuch as the chemical changes were taking place constantly due to the bacterial organisms which were reducing the sulphates and the organisms which oxidized the sulphureted hydrogen. It is quite certain, therefore, that chemical analyses taken at other periods would have shown a different relationship existing in the quantities of sulphates contained in the cultures of these various woods. For instance, in the culture of *Larrea tridentata* the evidence of the evolution of hydrogen sulphide preceded that in the other culture. An analysis gotten at the time the supply of hydrogen sulphide was especially abundant in the *Larrea* culture would certainly have given somewhat different results from those indicated in the above table for *Larrea tridentata.*

Chemical evidence shows that whenever an appreciable quantity of hydrogen sulphide is present in water whose sulphates have been acted upon by bacterial organisms, the reduction of sulphates has taken place and the decrease in the amount of sulphates is approximately proportional to the increase of the hydrogen sulphide.

Since the *Beggiatoa* lays hold of the hydrogen sulphide for energy releasal and oxidizes the sulphide, giving rise to acids, the question arises, might not any break-down of woody tissue present in a culture where this process was going forward be due to the presence of newly formed acids? This question was disposed of by the fact that the quantity of acids is very small and is immediately cared for by bases present in the water. Therefore, interesting as are these chemical changes, due to the reduction action of *Spirillum desulphuricans* and the oxidizing action of *Beggiatoa,* they can not be held to explain the decorticating changes observed in the wood sections placed in cultures of Salton Sea water.

## DECORTICATING PROCESSES IN SPECIMENS OF FRESH WOOD IMMERSED IN SALTON SEA WATER.

If the substances in the water of the Salton Sea, either before or subsequent to the processes carried on by the organisms above mentioned, were not active in producing decortication, there is only one possible avenue of investigation open in search for the causal factors of cortex removal.

Certain enzyme-producing bacteria would in all probability have access to the tissues which were decomposed. Investigations with reference to these forms were instituted in two directions; first, a series of controlled cultures were prepared consisting of three sets: A, a series of sections from *Larrea tridentata, Prosopis glandulosa,* and *Prosopis pubescens* were sterilized and placed in cultures of unsterilized Salton Sea water; B, sections of the same woods unsterilized were placed in cultures of sterilized water; C, sections of the same woods were sterilized and placed in cultures of sterilized Salton Sea water. These cultures were kept at room temperature and series A and B soon gave evidences of bacterial activity. The changes in B series were considerably greater than those in A, while series C remained inactive. Throughout the whole test it was noted in the sections from series A and B, after three weeks of culture, that the cortex and phloem loosened and slipped from the woody cylinder with ease.

In addition to the controlled cultures, sections of fresh *Prosopis glandulosa* were made in transverse, longitudinal, and tangential sections and placed in a 5-gallon carboy. Also specimens of branches 15 to 25 c.c. long were immersed in the same gross culture. Some of these branches were paraffined at the end, so that no bacterial organisms could secure a portal of entry to the cortex except through a superficial break in the epidermis. Other specimens were broken in such a way as to leave a ragged and exposed surface at the ends, giving the most ready access to the cortex, phloem, and cambium. Similarly 5-gallon carboy cultures were made of *Prosopis pubescens* and *Larrea tridentata.* These gross cultures kept under observation for six months gave distinct results with reference to decortication

wherever a portal of entry to the tissues of cortex, phloem, and cambium had been provided. On the contrary, the specimens whose ends had been protected by paraffine caps possessed a firm, close cortex and phloem which adhered as tightly to the woody cylinder five months after the cultures were made as when the specimens were first immersed.

The various stages of progressing decortication are indicated in Plates 13 and 14. In the latter stages the effects of decortication are pronounced, notably in *Larrea tridentata* (Plate 14, P, Q, O.) Moreover, it is evident that wherever the branches had sharp and even sections (Plate 13, B, C, G, H, I, and Plate 14, transverse sections of N, O, P, Q), the progress of decortication was far less rapid than in those branches which were broken at an angle producing ragged, jagged fractures, which furnished an admirable portal of entry to any bacterial organisms present in the culture. (Plates 13, D and E, and 14, N, O, P, and Q.)

In order to make microscopical investigations of the changes in the immersed specimens, sections of the various woods were prepared. Different methods of technique were employed, but the most successful results were secured when specimens of wood were softened for three weeks in hydrofluoric acid, thoroughly washed and imbedded in gelatine, hardened in formaline, and sectioned in a swinging microtome, stained 36 hours with safranine followed by a very brief treatment with aniline blue. Studies of these woods showed that the tissues of the woody cylinder were unchanged, likewise the lignified regions in the phloem were unaltered. (Plate 13, F and K, and Plate 14, R.) A distinctly different condition was present in unlignified parenchyma zones which alternated with the lignified zone in the phloem; and a notably disintegrated state of affairs was found in the region of the cambium. The walls of the meristem were totally dissolved in places and disintegration following the hydrolysis of these delicate walls had progressed so far that the slightest disturbance caused a breaking away of the whole cambial region from the woody cylinder, marking, of course, a completion of the decorticating process (Plate 13, D and E, and Plate 14, P, Q, and O). The two factors entering into this lack of uniform decortication are the number of layers of cambial cells intervening between the phloem and xylem and the degree of accessibility afforded to the agents which might act upon the walls of the cambial cells. This, in a measure, would explain why some trees, submerged for five years in the Salton Sea, were found to have patches of cortex remaining after they were exposed by the lowering of the water level.

The breaking down of the walls in the zone of the cambium and in the meristematic regions of the unlignified zones in the phloem (see Plate 13, D and E, and Plate 14, P and Q), can not be accounted for merely by reason of their having more delicate structure than that which characterizes the cells in the woody cylinder and in the lignified zones of the phloem. It is true that it would be possible to rend delicate tissues of this character if considerable physical force were applied to external parts of the uninjured specimens. Such an explanation, however, does not fit this case of decortication. There is a difference not only in the strength of the cell walls in these different regions, but there is a difference in the chemical composition of the walls of the cells of the cambium, the woody cylinder, and the lignified regions of the phloem. This was set forth by Mangin[1] who held that the cellular membrane in young tissues differed from that in adult tissues. He definitely denied that cell walls were composed of pure cellulose. He affirmed that they were always associated with groups of pectins which are essentially distinguishable by color reactions and by certain optical properties, and by great mutability under the action of acids and bases. After conducting extended researches, he concluded that tissues might have their cell walls disassociated by four different processes: (1) prolonged boiling in pure water; (2) prolonged boiling in a 2 to 5 per cent solution of caustic soda or caustic potash; (3) continued action in a cold, weak acid and solvents of pectic acid, alkalines, alkaline salts, ammonia water, and organic acids; and (4) it was possible that certain organisms, which

[1] Mangin: Jour. de Bot., vol. VII, p. 336, 1893.

## Details of Plate 14.

Figs.

L. *Larrea tridentata* submerged 1906 to 1908. Only the woody cylinder was left in the specimen submitted for investigation.

M. Carboy neck showing filaments of *Beggiatoa* in great abundance. This culture was two weeks old, having developed in the carboy shortly after specimens of fresh *Larrea* wood had been immersed in the carboy of Salton Sea water.

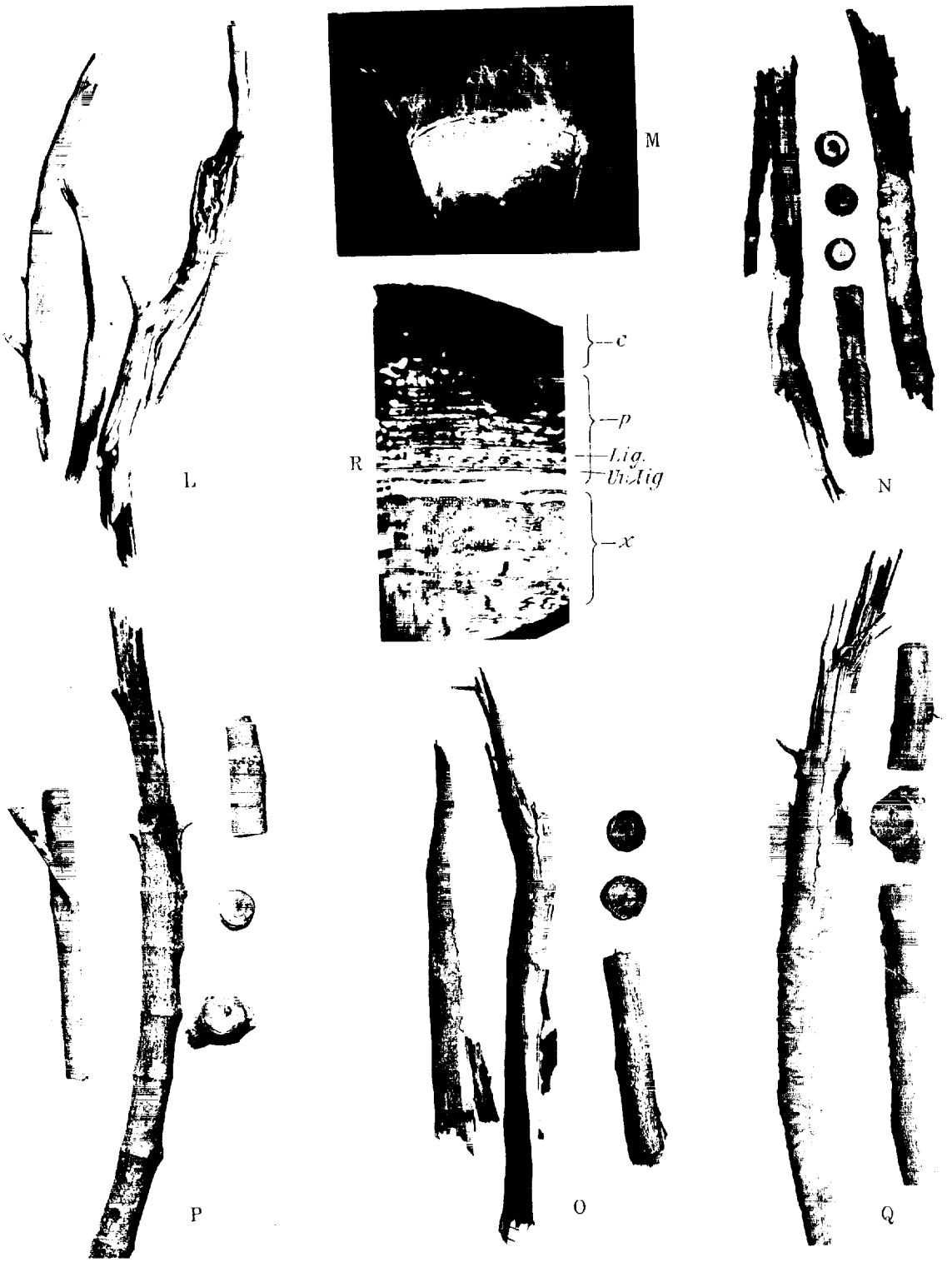
N. *Larrea tridentata* after two months' submergence in Lake Michigan water. Considerable decortication is shown in the long sections.

O. Culture shown in N two months later, when decortication has advanced far in the long sections whose ends had been twisted prior to submergence.

P. *Larrea tridentata* two months after being submerged in Salton Sea water. The twisted portions of the long specimens show great decortication, while the transverse sections are entire, and there is no breaking away of the cortical zone.

Q. Culture P two months later, when the decorticating process has advanced further. The transverse section has begun to break away in the cambial region after four months of Salton Sea submergence.

R. *Larrea tridentata* showing cortex (*c*), phloem (*p*), and xylem (*x*). The unlignified zone (*unlig.*) here is very wide, and the lignified zones are less numerous than in *Prosopis glandulosa* or *Prosopis pubescens*. This is closely associated with the different types of decortication indicated in D, E, I, and J of plate 13, and N, P, O, and Q of plate 14.




Larrea tridentata in different Stages of Decortication, Transverse Section of Fresh Non-immersed Stem. and also a Culture of Beggiatoa. The Water Cultures were maintained at Room Temperature, 22° C.

nourish themselves upon pectic compounds, could effect a disassociation of tissues. In this group of organisms he included a group of bacteria known as the Amylobacter group.

Mangin in this last conclusion was supported by previous workers, van Tieghem[1] and Trecul.[2] Louis Gaucher[3] held that the cell membranes were degenerated through the action of bacteria bringing about what he termed an abnormal pathological condition. Speaking of the relation of gums and pectin,[4] he states that a member of the Leguminosæ family, *Acacia vereck*, breaks down the walls of cells, disengaging all the vascular parts of the plant. In that case the pectin would be hydrolyzed by enzymes produced within the tissues of the growing plants. In other cases he quotes Mangin, who claimed that gum might be made to appear in the cortical parts of plants by injuring their branches with repeated blows upon the bark. In discussing these phenomena, however, these investigators note that the cell walls are composed of a series of complex carbohydrate compounds including pectic acid, pectose, pectin, and cellulose. They note that these substances may be modified in various ways through abnormal and normal processes. But in every case the modification is brought about either through action of a hydrolyzing agent produced entirely by the cells of the living plant itself, sometimes in a normal and sometimes in an abnormal condition, or else by the presence of invading organisms such as the *Amylobacter* which had gained access to the tissue whose cell walls had not become wholly lignified but were in the early stages of being laid down.

Subsequent to the completion of the experimental work with the fresh woods received from the Salton Sea region, it was suggested by Professor Joseph S. Caldwell that the decortication processes were similar to those involved in the retting of flax hemp. This suggestion was supported by an examination of the work of M. S. Winogradsky,[5] who carried on numerous experiments and investigations with waters concerned with the mass erosion of vegetable tissues. In these researches he came to the conclusion that he had to deal with a specific bacterial organism which he isolated in his fermentation experiments. This organism was apparently widespread and, according to Winogradsky, might be looked for in any waters where the successful retting of flax was carried forward.

In a later work, Professor Dr. J. Behrens investigated the retting of flax and hemp.[6] Professor Behrens, by means of extensive laboratory experiments, opposed the view of Hauman, namely, that the retting of flax and other vegetable tissues could be carried on successfully by many different species of bacteria. In a table containing a report upon a series of controlled cultures, he itemizes the organisms which would be able to produce so-called retting of flax and hemp. His general conclusions were that this work should be referred to definite and specific organisms.

Some of the best work in investigating cellulose fermentation was carried on by W. Omelianski.[7] His investigations were concerned with several phases of cellulose fermentation, such as the hydrogen fermentation of cellulose, the methane fermentation of cellulose, and the fermentation of cellulose through the dentrifying bacteria, aërobic bacteria, and mold fungi. Following his suggestions, it was possible to isolate an organism which grew upon sterile filter paper, which was apparently hydrolyzed by its action. In harmony with Omelianski's findings, this organism, which was isolated from Salton Sea water, required a long time for its incubation upon cellulose. The solution employed was composed of phosphate of potassium 1 gram, magnesium sulphate 0.5 gram, sulphate of ammonia 1 gram; a very small quantity of sodium chloride in a liter of distilled water used as a solvent. Steril-

[1] van Tieghem, Ph., (1) Comptes rend. de l'Acad., 1879, vol. 88, p. 205. (2) Also vol. LXXXIX, pp. 25 and 1102, 1879. (3) Bull. de la Soc. Bot. de France, vol. XXIV, p. 128, 1877; vol. XXVI, p. 25, 1879; also vol. XXVIII, p. 243, 1881.
[2] Trecul, A., (1) Comptes rend. de l'Acad., vol. LXI, pp. 156 and 436, 1865; also vol. LXV, p. 513, 1867.
[3] Gaucher, Louis: Etude générale de la membrane cellulaire, 1904.
[4] Gaucher, Louis: Ibid., p. 207, 1904.
[5] Winogradsky, M. S., Sur le rouissage du lin et son agent microbien. Comptes Rendu, vol. CXXI, p. 742, 1895.
[6] Behrens, J., Ueber die Taurotte von Flachs und Hanf. Parasitenkunde und Infektion krankheiten, vol. X, pp. 524–530, 1903.
[7] Omelianski, W.: See note 3.

ized strips of filter paper were then placed in this solution, and flasks containing these preparations were inoculated with 10 to 50 c.c. of water from the different cultures of the Salton Sea woods. These were incubated at room temperature and, of course, accompanied by controls. At the expiration of a week's time, a slight cloudiness was observed in the inoculated cultures. Within a few days small colonies were observed growing on the filter paper. These conformed in essential respects to the group of organisms described by Omelianski as capable of carrying on hydrolysis of cellulose. They were isolated most satisfactorily from the culture of Salton Sea water containing *Prosopis glandulosa*. There was every evidence that successful cultures depended upon using large quantities of the inoculating fluid, though it is probable that small pieces of the infected wood might have been far more effective as an inoculating agent than was the water in which the wood was immersed.

In view of the findings of the authorities quoted there seems to be no question that the disintegration of cambial cell walls and consequent removal of the cortical and phloem portions of woody plants submerged in brackish waters is to be attributed to the action of bacterial organisms belonging to the *Amylobacter* group. From this it would seem that the present problem and the mass erosion carried on by substances present in the water where flax and hemp are retted are related to such economic problems as the breaking down of cellulose or its related compounds when they pass through the digestive tract of animals, or through the septic tank of sewage-disposal plants, and also to the ultimate breaking down of the mantle of humus overspreading the earth.[1]

## SUMMARY.

1. Woody plants submerged by the flooding of the Salton Sea were found to be decorticated after a period of one year.

2. Microscopical preparations of samples of fresh woods placed in Salton water and kept under control were found breaking down in the zone of meristematic cells, notably in the region of the cambium and in the zones between lignified regions of the phloem.

3. The chemical composition of Salton water could not account for the decortication of woody plants submerged in the sea for a period of one to five years.

4. Sterilized specimens of fresh woods placed in sterilized Salton water did not decorticate during the ten months they were kept under inspection.

5. Bacteriological cultures made it possible to isolate the bacterial organisms, belonging to the *Amylobacter* group, which produce an enzyme capable of hydrolyzing pectins which are considered by some chemists to be the principal substances in young cell walls.

6. A microscopical study of woods which emerged annually from the autumn of 1907 to 1911 did not show breaking down of cell walls in any portion of the wood cylinder. Cell walls of the same tissue in the woody specimens of all the species examined were characterized by the same general thickness.

Consequently, it is believed that the action of the Salton Sea water on tissues of woody plants is wholly related to hydrolyzing agents having a bacterial origin; and, furthermore, evidence is lacking that petrifaction had begun in the tissues of the woody plants submerged for four years in the Salton Sea.

It is a pleasure to make grateful acknowledgment of the helpful suggestions received from Prof. John M. Coulter and Dr. W. J. G. Land during the time that these investigations were continued. I am especially indebted to Dr. Land for valuable directions in developing technique suitable for making microscopical preparations of the bone-hard specimens of dead woods which were received from the Salton Sea for study.

[1] While reviewing the above manuscript for publication, Bulletin 266 from the Bureau of Plant Industry of the U. S. Dept. of Agriculture was received. This bulletin reports results obtained by Physiologist I. G. McBeth and Assistant Soil Mycologist F. M. Scales, while they were investigating "The Destruction of Cellulose by Bacteria and Filamentous Fungi." In determining the work of cellulose-dissolving bacteria they made "examinations of sewer slime, of manures, and of the soils of the United States." Apparently their studies are allied to, but in no sense identical with, the Salton Sea problems of cellulose hydrolysis.